Cochlear Potentials in the Rhesus and Squirrel Monkey

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The cochlear potentials were studied in rhesus and squirrel monkeys. Contrary to previous reports, the data revealed no important differences between the results obtained in these animals and those reported for the cat and guinea pig. Round-window recording of responses to sound stimuli exhibited cochlear microphonics of nearly 2 mV at maximum. The input–output curves showed that CM of these primates behaved not differently from those reported for the cat and guinea pig. When a micropipette was advanced from scala tympani into the organ of Corti, an increase in magnitude of CM was recorded, as well as a negative dc potential of about 75 mV. Perforation of the reticular lamina was associated with a change in polarity of CM and the appearance of an endocochlear potential (about +75 mV). Oxygen deprivation depressed CM and changed the polarity of the endocochlear potential from +75 to −20 mV. A comparative anatomical study showed that the cochleas of primates, cat, and guinea pig are similar.

MOST of the information on the electrophysiology of the cochlea has been obtained from studies where guinea pigs and cats were used as the experimental animals. There are good reasons for using these particular species. The main reason for working with the guinea pig is that each turn of the cochlea is accessible for inserting electrodes. A prime advantage in using the cat is that the round-window membrane can be exposed easily and an electrode can be readily placed upon it.

It is reasonable to assume that the cochleas of all mammals perform similarly, and indeed the assumption is implicit in our theories of hearing. The experimental data from the monkey and man, however, might cause one to hesitate before accepting unconditionally the generality of any theory of cochlear action which is largely based on the data from rodents and carnivores. More specifically, the discrepancy between the data obtained in primates and those recorded for other mammals centers upon the amplitude of the cochlear microphonics (CM). CM in the primate appears to be significantly smaller, and the size of CM bears directly on the question of what is the excitatory process which initiates the nerve impulse. If the nerve endings are excited electrically, then CM may be regarded as the sign of electric stimuli acting upon the nerve endings.

From the papers of Lempert *et al.*,[1,2] the CM amplitude of the rhesus monkey appeared to be no more than 25 μV as recorded from the round window. In relative terms, this is indeed quite small when one considers that the CM in the guinea pig recorded from the same location can be larger than one millivolt. More recently, Wever, Vernon, and Lawrence,[3] recording from the round window of the rhesus monkey, reported CM where amplitudes were as high as 400 μV. Whereas these magnitudes are appreciably greater than those recorded in the Lempert *et al.*[1,2] study, they are still less than those normally found in rodents and carnivores. Wever *et al.*[3] suggested that the relatively small CM in the monkey may, in part, be due to a partial short-circuiting of the potentials brought about by a greater conductivity of the bone surrounding the primate cochlea. This explanation is equally applicable to the situation in

[1] J. Lempert, E. G. Wever, M. Lawrence, and P. E. Meltzer, "Perilymph: Its Relation to the Improvement of Hearing Which Follows Fenestration of the Vestibular Labyrinth in Clinical Otosclerosis," Arch. Otolaryngol. **50**, 377–387 (1949).

[2] J. Lempert, P. E. Meltzer, E. G. Wever, and M. Lawrence, "The Cochleogram and Its Clinical Application," Arch. Otolaryngol. **51**, 307–311 (1950).

[3] E. G. Wever, J. A. Vernon, and M. Lawrence, "The Nature of the Cochlear Potentials in the Monkey," Acta Oto-Laryngol. **49**, 87–92 (1958).

man, where again the maximum amplitude of CM is inordinately small, even when allowance is made for the adverse conditions of recording which usually have characterized these studies.[2,4–9]

The purpose of the present experiment was to record cochlear potentials from the monkey with the recording electrode in scala media. Hence, we sought to find out whether the CM amplitude remained small when the electrode was placed in a region where large CM amplitudes have been repeatedly recorded in other animals. In addition, our experimental conditions and recording arrangements were set up so that we could record other cochlear potentials. Of particular interest was the endocochlear potential (EP) discovered by Békésy[10] in the guinea pig. He demonstrated that the endolymph near the round window was +80 mV relative to the perilymph. It is implicit in the writings of Békésy[10,11] that EP serves as the energy source of CM. Also, we wish to determine whether the EP of the monkey changes from positive to negative values under conditions of oxygen deprivation, as Békésy[11] showed previously in the guinea pig. In addition to recording CM and EP, we arranged to record the summating potential (SP)[12] and the negative dc potential in the organ of Corti[10] so that these could be compared with the findings in the guinea pig. Lastly, round-window recordings were also employed in order to compare our data with those of others.

METHODS

The experiments were carried out in four adult squirrel monkeys (7 ears were examined) and five adult rhesus monkeys (9 ears). The animals were anesthetized with pentobarbital sodium, 37 mg per kg body weight injected intraperitoneally. Immediately thereafter, a tracheotomy was performed.

Surgical Procedure

The pars mastoidea and stylomastoid foramen containing the facial nerve were exposed by a retroauricular incision. The mastoid cells were removed along the facial nerve. When this procedure is followed, the removal must be extensive, but care should be taken when the mastoid cavity is expanded above the facial canal, because the ring of the tympanic membrane may be damaged or the external canal be penetrated. For exposing the round-window niche, a hole was made into the middle-ear cavity passing through the descending segment of the facial nerve. Enlargement of this hole exposed the round-window membrane through which a segment of the basilar membrane and spiral lamina could be seen under a dissecting microscope. The exposure permitted placing a gross electrode on the surface of the round-window membrane or directing a micropipette through it into the organ of Corti and scala media.

Recording Procedure

The electrode employed for recording from the round window consisted of an enameled silver wire (100-μ diameter) whose tip was flattened and the insulation removed. The electrode was mounted on a micromanipulator, and the tip was placed on the central area of the round-window membrane. The bony defect was not closed so that in all experiments the middle-ear cavity remained open. The round-window niche was kept free of blood and fluid. Accumulation of fluid in this region usually was associated with a considerable decrease in amplitude of responses to sound stimuli.

Glass micropipettes were used for recording from organ of Corti and scala media. Pipettes of 0.5 μ in tip diameter (resistance 35 MΩ) and of 1–2 μ (resistance 5MΩ) were filled with 3 *M* KCl. Pipettes of larger diameters (3–10 μ) filled with mammalian Ringer were also used. The micropipette housed a silver-chloride electrode which was connected to a cathode-follower stage. The responses to sound stimuli were observed or photo-

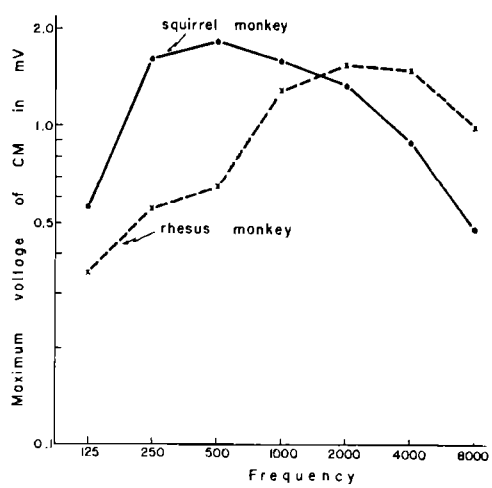

FIG. 1. Maximum voltage of cochlear-microphonic response in monkeys plotted as a function of sound frequency. The figure represents the measurements for one rhesus and one squirrel monkey.

[4] J. Lempert, E. G. Wever, and M. Lawrence, "The Cochleogram and Its Clinical Application. A Preliminary Report," Arch. Otolaryngol. **45**, 61–67 (1947).

[5] B. Fromm, C. O. Nylén, and Y. Zotterman, "Studies in the Mechanism of the Wever–Bray Effect," Acta Oto-Laryngol. **22**, 477–486 (1935).

[6] A. M. Andreev, A. A. Arapova, and S. V. Gersuni, cited by Lempert *et al.*[4]

[7] H. B. Perlman and T. J. Case, "Electrical Phenomena of the Cochlea in Man," Arch. Otolaryngol. **34**, 710–718 (1941).

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[9] R. J. Ruben, J. Sekula, J. E. Bordley, G. G. Knickerbocker, G. T. Nager, and U. Fisch, "Human Cochlear Responses to Sound Stimuli," Trans. Am. Otol. Soc. **48**, 52–72 (1960).

[10] G. von Békésy, "DC Resting Potentials inside the Cochlear Partition," J. Acoust. Soc. Am. **24**, 72–76 (1952).

[11] G. von Békésy, "Gross Localization of the Place of Origin of the Cochlear Microphonics," J. Acoust. Soc. Am. **24**, 399–409 (1952).

[12] H. Davis, C. Fernández, and D. R. McAuliffe, "The Excitatory Process in the Cochlea," Proc. Natl. Acad. Sci. U. S. **36**, 580–587 (1950).

graphed with a double-beam oscilloscope. The dc potentials were displayed on the oscilloscope or recorded with an Offner Dynograph. The ground electrode was a large silver wire imbedded in Ringer-agar gel and was placed on the neck muscle of the animal.

Sound Stimuli

The input–output function of CM was determined by using pure tones ranging from 125 to 9400 cps. Maximum outputs of CM and SP were also measured with tone bursts at several frequencies. The sound stimuli were delivered through a plastic tube fitted to the external ear canal in some animals, while in others a free-field technique was used.

In a few monkeys, the behavior of cochlear responses to oxygen deprivation was observed. For this purpose, the monkey was curarized and maintained under artificial respiration. Fulminating anoxia was induced by clamping the tracheotomy tubing.

Anatomical Studies

Two squirrel monkeys were sacrificed at the end of the experiment by intravital perfusion with Heidenhain–Susa fixative solution. The temporal bones were removed and prepared for studying the cytoarchitecture of the organ of Corti and measuring the length of the basilar membrane. A corresponding study was done with a collection of temporal bones from the rhesus monkey. A comparative anatomical study was made of the cochleas of the monkey, cat, and guinea pig.

RESULTS

The results showed that the CM amplitude in monkey has a much higher value than that previously reported. Moreover, the summating potential, the negative dc potential of the organ of Corti, and the endocochlear potential were essentially no different from those obtained from guinea pigs under comparable experimental conditions.

Round-Window Recordings

Figure 1 shows the maximum output of CM for frequencies ranging from 125 through 9400 cps. The data for the rhesus monkey are in agreement with those reported by Wever *et al.*[3] in that the CM magnitudes are greatest when the stimulus is in the neighborhood of 2000 cps. We observed CM, however, where amplitudes were more than four times those recorded by other investigators. It should be mentioned that the CM amplitudes of the rhesus monkeys used in the present study were lower for most of the frequencies than the CM amplitudes shown in Fig. 1. Nonetheless, the most valid estimate of the CM amplitude for any given species is the highest that can be recorded, provided, of course, there be no reason to suspect the presence of an artifact.

Cochlear microphonics of almost 2 mV were recorded in the squirrel monkey. An interesting aspect of these data on the squirrel monkey is that a stimulus of relatively low frequency produces the largest CM amplitudes. Round-window electrodes are known to record primarily the electrophysiological events that occur in the basal turn of the cochlea. And, at least in the cat and guinea pig, CM amplitudes at the round window for the low frequencies are smaller than those elicited by stimuli of intermediate frequencies. This may mean that the basal turn of the squirrel monkey's cochlea is relatively more responsive to low-frequency signals.

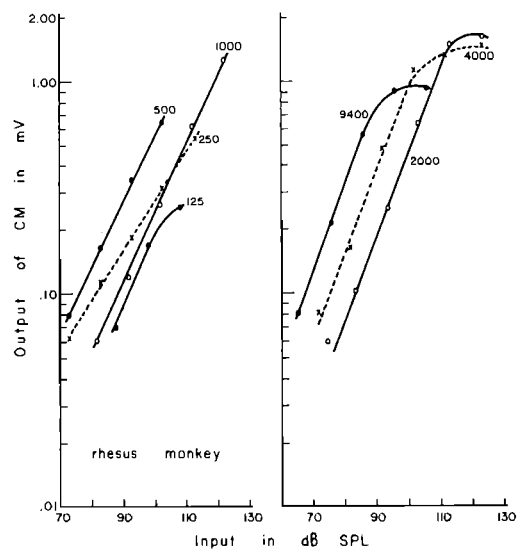

FIG. 2. Input–output curves of CM as recorded from the round window of a rhesus monkey. Notice that, in this animal, the point of nonlinearity of CM for low frequencies was not reached.

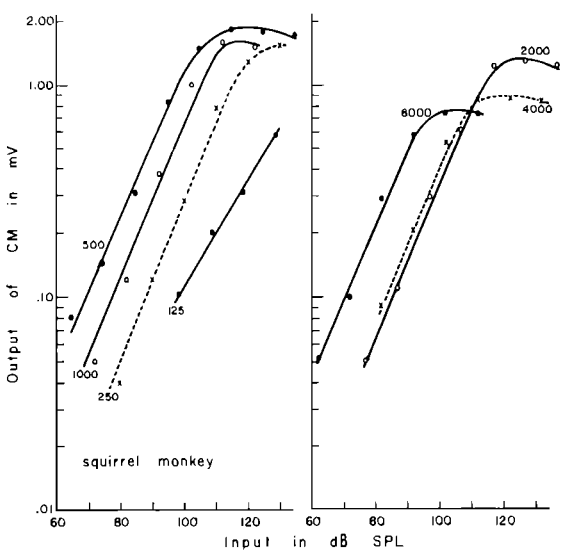

FIG. 3. Input–output curves obtained from round-window recording in a squirrel monkey.

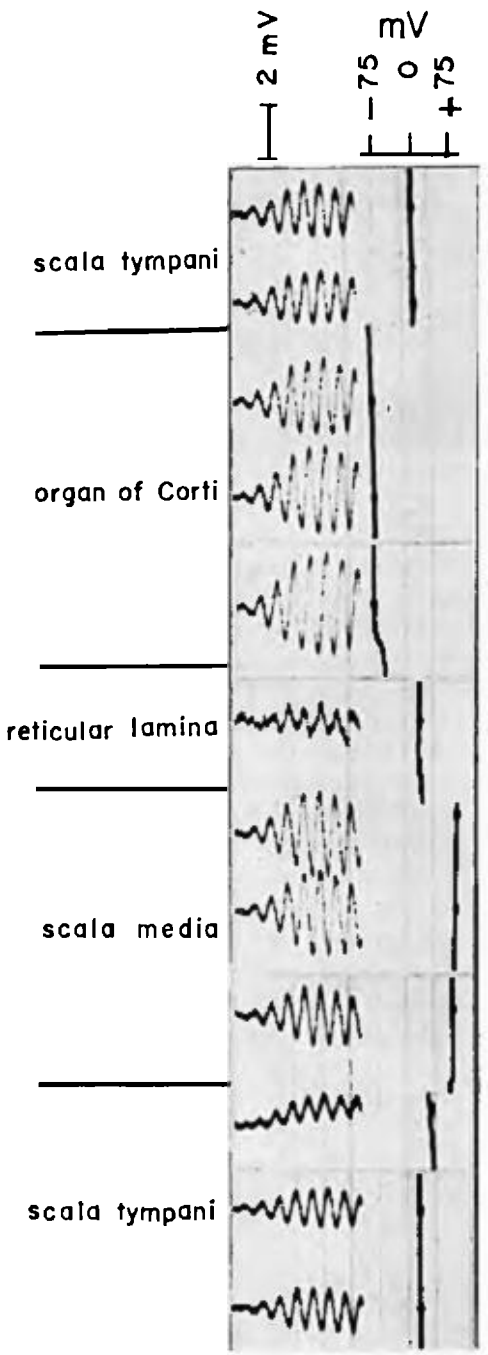

FIG. 4. Changes in CM and dc potential as a micropipette was advanced from scala tympani to scala media. Shown in the left column are the records of CM, and in the right column are shown the simultaneous recording of changes in the dc potential. Calibration is at the top of each column. The excerpts are to be read from top to bottom as they represent the sequence of events as the micropipette was advanced into scala media and then withdrawn. The positions of the recording tip given in the figure were inferred from the observed potentials. In scala tympani, the amplitude of CM is about 2 mV, and the dc potential relative to neck is zero. Penetrating the organ of Corti resulted in an increase in CM voltage to 3.5 mV and EP of +75 mV appeared. When the pipette was withdrawn to scala tympani, the CM amplitude and phase reverted to that originally observed, and the DC potential returned to zero.

Data from the same rhesus and squirrel monkeys are again shown in Figs. 2 and 3, where the input output functions for low-, intermediate-, and higher frequency stimuli are presented. The curves demonstrate that the pattern of the functions is similar to those reported in other mammals. In regard to the rhesus-monkey data, CM at the lower frequencies was still increasing when no further increments in the stimulus intensity could be attained with our equipment. This implies that the maximum CM amplitudes for low frequencies (see Fig. 1) are in reality greater.

Scala-Media Recordings

When, in the rhesus monkey, a glass micropipette was inserted through the round window, basilar membrane, and into scala media, CM as large as 2 mV was recorded. The stimulus in this case was a 720 cps tone burst whose intensity was 100 dB SPL. CM amplitude of 3.5 mV to a 1500 cps tone burst was recorded in the squirrel monkey. Shown in Fig. 4 are excerpts from a continuous recording of CM in this animal when a micropipette was advanced from the round window to scala media. Aside from the large CM amplitudes, it is of theoretical interest that the phase of CM changed by 180° when the micropipette traveled from organ of Corti to scala media. Similar observations on guinea pigs were instrumental in fixing the site of CM generators at the reticular lamina which articulates with the hair bearing end of hair cells.[13,14]

The summating potential in the squirrel monkey, measured in the basal turn of the scala media, was also prominent. When the intensity of a 7400 cps tone burst was increased from 71 to 111 dB, SP amplitude increased from 0.2 to 2.0 mV. Like CM, the phase of SP (and, hence, its polarity) reversed when the recording electrode was advanced from organ of Corti into scala media An SP of 700 μV was observed in scala media of a rhesus monkey to a 9400 cps tone burst delivered at about 31 dB SPL.

Penetration of the basilar membrane with the micropipette consistently gave a negative dc potential of the order of 75 mV. Often, this negative potential in the region of the organ of Corti could be recorded for only a few seconds, but several times it was maintained for 20 sec or more. The tip diameter of the micropipettes used ranged from 0.5 to 8 μ.

The endocochlear potential for all monkeys ranged from +65 to +75 mV at the start of scala media measurements. After a while, these values could decrease, presumably because of damage to the cochlear duct. The magnitudes of the recorded EP were slightly less than those normally found in the guinea pig, but no significance need be attached to these observed differences.

[13] H. Davis, "Biophysics and Physiology of the Inner Ear," Physiol. Rev. 37, 1–49 (1957).
[14] I. Tasaki, "Hearing," Ann. Rev. Physiol. 19, 417–438 (1957).

As reported for the guinea pig,[15] the polarity of the EP reverses during anoxia. Figure 5 illustrates this phenomenon in the rhesus monkey. EP attains a negative value after three minutes of asphyxia, but, upon reintroduction of oxygen, EP promptly reaches its former size.

Anatomical Studies

The cochlear receptor of rhesus and squirrel monkeys, guinea pig, and cat exhibited under light microscopy a similar cytoarchitecture. However, on the basis of the shape of organ of Corti and dimensions of structures, each species can be differentiated from the others (Fig. 6).

The length of the basilar membrane was determined by reconstructing the cochlea according to the method described by Guild.[16] Since the height of the cochlea is not considered in this method, the measurements were corrected by a factor of 2.8%, as determined by Schuknecht.[17] In four specimens of the squirrel monkey, the mean length of the basilar membrane was 20.4 mm, the length ranging from 19.6 to 21.0 mm. In four rhesus-monkey cochleas, the mean length was 23.3 mm, the length ranging from 22.9 to 23.6 mm.

These measurements are roughly comparable to data given for the cat and guinea pig: 22.0 mm[17] and 18.8 mm,[18] respectively.

DISCUSSION

When recordings are made from the round window, organ of Corti, and scala media of the rhesus or squirrel monkeys, maximum voltages of CM can be obtained which are comparable to those described for the guinea pig and cat.[19,20] The patterns of input–output functions of these four species are similar. In the rhesus monkey, however, the curves for some low frequencies were linear up to the maximum sound intensity provided by our instruments. This observation confirms the finding of Wever *et al.*,[3] who reported that for rhesus monkey the point of maximum output of CM occurs at higher sound-pressure levels than that of guinea pig or cat. The reason for this is obscure.

It is apparent from examining the anatomy of these four species that the cochleas are highly similar. Yet, they are by no means identical. There are differences in population and morphology of certain cells and in the shape and dimensions of some structures. These distinctions may account for certain variations in cochlear function, such as differences in frequency range and maximum sensitivity which are reported to exist from one species to another.[21]

The findings reported here are important for the theory of cochlear action which favors CM as playing a critical role in the initiation of nerve impulses. The feasibility of this action and, hence, the validity of the theory were called into question when the CM for the primate appeared to be only 0.4 mV in contrast to the cat and guinea pig, where CM of 1.0 mV or more was consistently found. Since we recorded CM voltages as large as 3.5 mV in primates, it is reasonable to assume that the sensory hair cells, as stated by Wever *et al.*,[3] behave in the same manner in different species.

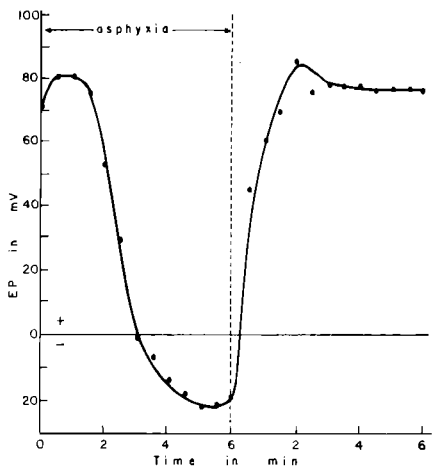

FIG. 5. This diagram illustrates the time course of the EP during and after a period of six minutes of asphyxia. During this interval, EP changes from +70 to −20 mV. With readmission of air; the potential returns rapidly, overshooting for a short time the pre-asphyxsic value.

The reason for the discrepancy between our measurements and those reported in the literature[1,3] is not clear. Our experiments were carried out in two different laboratories (The University of Chicago and National Institutes of Health), using different stimulating and recording equipment; nonetheless, the results were comparable. Perhaps the discrepancy in findings between this study and earlier ones may be attributed to differences in surgical procedures. We wish to emphasize that the surgical procedure in 14 out of 16 ears was carried out by one surgeon who also supervised the operation in the other two ears. The approach was the same for all animals. Furthermore, it seems quite relevant to our thesis that in two ears, not reported here, damage to the tympanic membrane or ossicular chain was associated with a considerable depression of all cochlear responses. These observations suggest strongly that the

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[16] S. R. Guild, "A Graphic Reconstruction Method for the Study of the Organ of Corti," Anat. Record **22**, 141–157 (1921).

[17] H. F. Schuknecht, "Techniques for Study of Cochlear Function and Pathology in Experimental Animals. Development of the Anatomical Frequency Scale for the Cat," Arch. Otolaryngol. **58**, 377–397 (1953).

[18] C. Fernández, "Dimensions of the Cochlea (Guinea Pig)," J. Acoust. Soc. Am. **24**, 519–523 (1952).

[19] I. Tasaki, H. Davis, and J. P. Legouix, "Space–Time Pattern of the Cochlear Microphonics (Guinea Pig) as Recorded by Differential Electrodes," J. Acoust. Soc. Am. **24**, 502–519 (1952).

[20] E. G. Wever, *Theory of Hearing* (John Wiley & Sons, Inc., New York, 1949).

[21] E. G. Wever, "The Cochlear Potentials and Their Relation to Hearing," Trans. Am. Otol. Soc. **47**, 13–27 (1959).

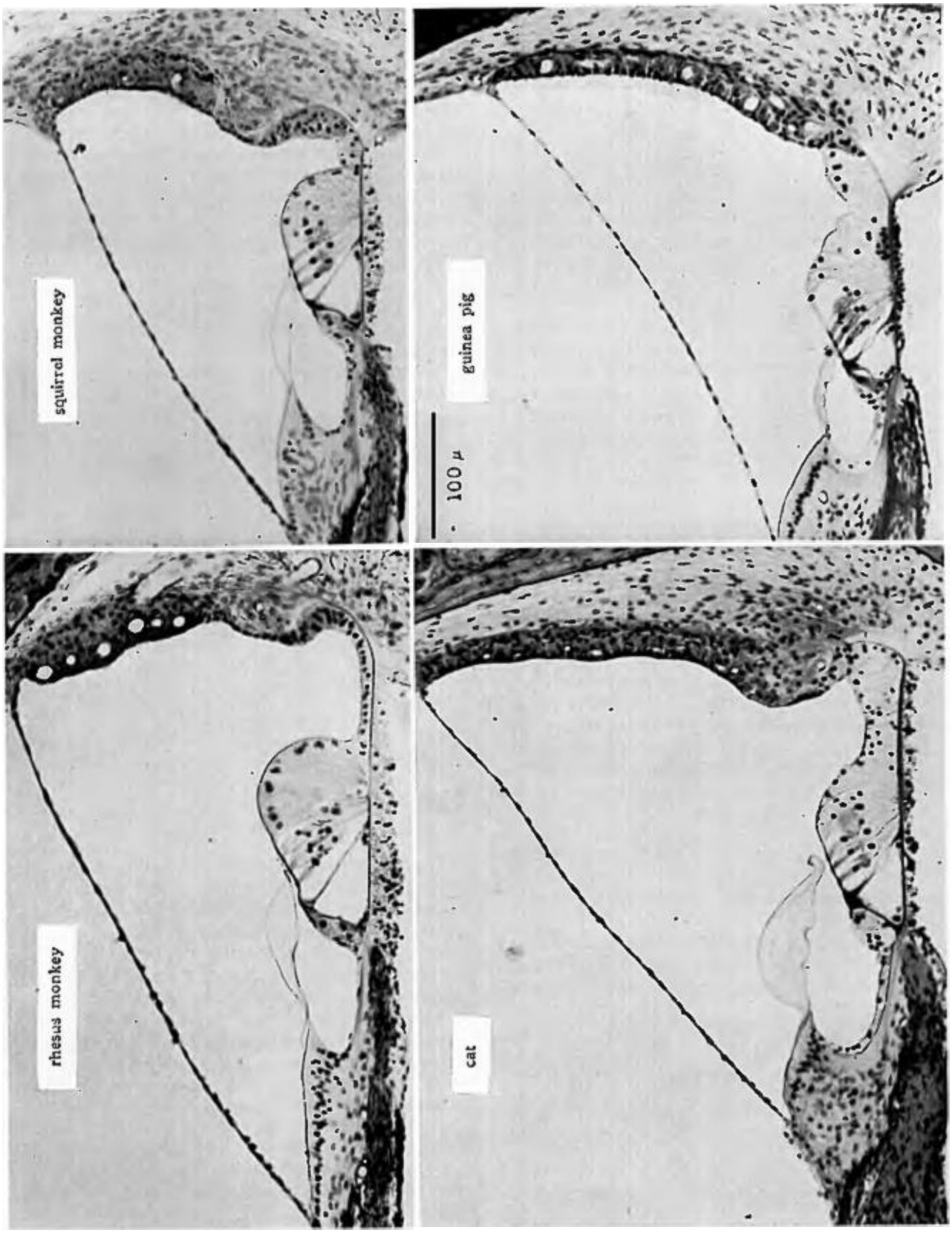

FIG. 6. Cytoarchitecture of the cochlear receptor in four species. Notice that all present similar types and distribution of cells, but shape and dimensions of structures vary from one species to another. Hematoxylin eosin stain. Marker 100 μ for all photomicrographs.

small amplitude of CM reported for man [2,4–9] may be caused by the fact that all subjects exhibited pathologic ears and/or that testing was done under adverse conditions.

Thus far, we have discussed only our findings on CM, but certainly of equal significance are our data on other cochlear potentials: organ of Corti dc potential, summating potential, and endocochlear potential. In the monkey, these potentials behave in a manner similar to that described for the cat and guinea pig.

The origin of the negative dc potential in the organ of Corti has not been clarified yet. Apparently, as observed in several species of therian mammals (marsupials and placentals), there are two negative dc potentials in the organ of Corti. One, corresponding to the classical intracellular potential, can be measured with a micropipette in various cells forming the organ of Corti, as shown originally by Békésy.[10] The other, measuring about −75 mV relative to the perilymph, can be recorded with a pipette of 15 μ or more. Because it can be recorded with such large electrodes, Tasaki and Spyropoulos[22] suggested that this potential was probably extracellular. Perhaps it is associated with the chemical composition of the fluid contained in the organ of Corti. This fluid is not endolymph as pointed out by Davis,[23] because it is not possible for naked nerve fibers to transmit impulses when embedded in a potassium-rich medium. Engström[24,25] believes that the tunnel of Corti, Nuel's space, and the spaces around the hair cells contain a fluid ("cortilymph") which is different from both endolymph and perilymph.

As in other mammals, the amplitude of CM and SP of the monkey was found to increase as the micropipette penetrated the basilar membrane. Both potentials changed in phase after the reticular lamina was penetrated, and a large positive dc potential comparable to that described by Békésy[10] in the guinea pig was recorded from scala media. The observations made to date in several species of therian mammals consistently reveal an EP of +75 mV or more. On the other hand, birds and reptiles exhibit an EP of +20 mV or less.[26] The significance of this difference for the sense of hearing among species is not immediately apparent.

The similarity of cochlear function among several mammals was also revealed by experiments on oxygen deprivation. In the monkey, the behavior of CM, SP, and EP under anoxic conditions followed the same pattern as that reported in the guinea pig,[11,15] including the change of EP from positive to negative values. The basis for the negative endocochlear potential observed during oxygen deprivation and for a few hours after death is not known. Békésy[11] suggested that this long-lasting negative dc potential is due to loss of insulating properties of Claudius or Hensen cells. However, recent evidence[15,27] suggests other possibilities, such as an accumulation of metabolites and/or the difference in the concentrations of ions between endolymph and perilymph.

ACKNOWLEDGMENTS

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[22] I. Tasaki and C. S. Spyropoulus, "Stria Vascularis as Source of Endocochlear Potential," J. Neurophysiol. **22**, 149–155 (1959).

[23] H. Davis, "Mechanism of Excitation of Auditory Nerve Impulses," in *Neural Mechanisms of the Auditory and Vestibular Systems*, edited by G. L. Rasmussen and W. F. Windle (Charles C Thomas, Springfield, Illinois, 1960), Chap. 2, pp. 21–39.

[24] H. Engström, "Electron Micrographic Studies of the Receptor Cells of the Organ of Corti," in *Neural Mechanisms of the Auditory and Vestibular Systems*, edited by G. L. Rasmussen and W. F. Windle (Charles C Thomas, Springfield, Illinois, 1960).

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[27] V. Honrubia, R. Butler, B. Johnstone, and C. Fernández (unpublished data).